DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

069149/
069208

1. **CHEMICAL:** didecyldimethylammonium chloride (DDAC) **PC Code No.:** ~~069149~~

2. **TEST MATERIAL:**[1] Bardac 2280 **Purity:** 81.0 % ai
ID No.: 100033 : 99.88% radiochemical purity

3. **CITATION**

Author:	Henry O. Krueger, Ph.D. (Study Director), D. Desjardins, T. Kendall, and R. Vanhoven
Title:	Bardac 2280: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum)* Using Natural Surface Water
Study Completion Date:	April 7, 2003
Laboratory:	Wildlife, International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
Sponsor:	Lonzagroup (Lonza Inc.) 17-17 Route 208 Fairlawn, New Jersey 07410
Laboratory Report ID:	Wildlife International, Ltd. Study No. 289A-153
DP Barcode:	D294163
MRID No.:	459074-01

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist, US EPA/OPP/AD/RASSB

Signature: Kathryn V Montague **Date:** 12/15/04

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader, US EPA/OPP/AD/RASSB

Signature: **Date:** 12/16/04

6. **STUDY PARAMETERS**

Definitive Test Duration: 96-hr

[1]Nonlabeled and radiolabeled forms used

2053626

Type of Concentrations: Nominal and Mean Measured (initial)

7. CONCLUSIONS

An algal toxicity test was performed on the freshwater alga, *Selenastrum capricornutrum*, using natural surface water. The effects of Bardac 2280 on algal cell density and area under the growth curve were determined for a period of 96 hours, during which the cell density in each test solution was measured using a hemacytometer and microscope every 24 hours. Effects were reported as EC_{10}, EC_{50}, EC_{90} and NOEC values based on cell density and area under the growth curve. The reported study results were based on the mean measured concentration of the test solutions on Day 0; the verified results are based on 96-h mean measured concentrations. The test substance had an algistatic effect. No deformed or abnormal cells were observed. In comparison to a concurrent study (MRID 458964-02) performed with standard algal medium, there was an approximate 6-fold increase in the 96-hr EC_{50} values from the river water algal medium. The Study Report states that river water algal medium does appear to mitigate toxicity.

96-hour results:

Cell Density (µg a.i./L)	**Reported (0 hour meas. conc.)**	**Verified (96h meas. conc.)**
96-hr		
EC_{10} (95 %CI):	<10 (NA)	9.64 (0 - 34.2)
EC_{50} (95 %CI):	151 (129 - 176)	73.2 (66.5 - 80.8)
NOEC:	82	27

8. ADEQUACY OF THE STUDY

A. **Classification:** Supplemental

B. **Rationale:** Study was submitted concurrently with a standard algal toxicity study using sterile water as supplemental data.

C. **Repairability:** N/A

9. GUIDELINE DEVIATIONS

- The study was conducted using the Wildlife International, Ltd protocol which is based on OECD Guideline 201, harmonized OPPTS Test Guideline 850.5400,

and EC Guideline L383A - C.3. The OECD and EC Guideline criteria may differ from the OPPTS Guideline (850.5400) that was used in preparing this Data Evaluation Record.

- The study was conducted in compliance with FIFRA Good Laboratory Practice Standards (40 CFR Part 160) with the exception that the characterization of the radiolabelled test substance was not determined in compliance with Good Laboratory Practice Standards.

- The reported initial test pH of 7.7 to 7.8 was higher than the recommended starting pH of 7.5 ± 0.1.

- The age of the stock culture was not provided.

- Photosynthetically active radiation not reported.

- An exploratory range-finding test was conducted; however, details were not provided in the Study Report.

- The primary stock of ^{14}C-DDAC was prepared in NANOpure® water rather than river water algal media. The Study Report states that this deviation had no apparent effect on the study.

- Total suspended solids were not determined for filter/sterilized river water algal media.

- The test chemical concentration declined over the study period, reportedly due to binding to the glassware used. The study report states that this "bound" chemical was still available to the test system, and used the 0-hour mean measured concentrations to determine statistical endpoints; however, **the Agency will use the verified results based on the 96-hour mean measured concentrations to determine endpoints.**

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • *Selenastrum capricornatum (Raphidocelis subcapitata)* • *Skeletonema costatum* • *Anabaena flos-aquae* • *Navicula pelliculosa*	• *Selenastrum capricornatum*
Initial Number of Cells • 10,000 cells/mL (*Selenastrum, Anabaena, Navicula)* • 77,000 cells/mL (*Skeletonema*)	• Approximately 10,000 cells/mL
Stock Culture • 3 to 7 days old	• Age not provided
Nutrients • Standard formula (ASTM E1218-20) • pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) • Freshly prepared	• Algal cells cultured and tested in river water algal medium. • Stock nutrient solutions prepared by mixing reagent-grade chemicals with purified well water. The test medium then prepared by adding stock solutions to purified river water. • pH 7.5 ± 0.1 (adjusted prior to use with 10% HCL and sterilized by filtration).

B. Test System

Guideline Criteria	Reported Information
Solvent • Upper limit - 0.5 mL/L	• Solvents were not used.

Guideline Criteria	Reported Information
Temperature • 24° ± 2°C (*Selenastrum, Navicula, Anabaena*) • 20° ± 2°C (*Skeletonema*) • Recorded hourly	• Approximately 23.2 to 24.2° C. • Measured continuously in the environmental chamber and recorded twice daily in a container of water adjacent to test chambers.
Light Intensity • 4.3 K lx (± 10%) (*Selenastrum, Skeletonema, Navicula*) • 2.2 K lx (± 10%) (*Anabaena*) • Photosynthetically active radiation approx. 66.5 ± 10% μEin/m^2/sec	• 3,860 to 4,730 lux (measurements taken at five locations surrounding the test flasks). • Photosynthetically active radiation not reported.
Photoperiod • 14-hr light/10-hr dark (*Skeletonema*) • Continuous (*Selenastrum, Navicula, Anabaena*)	• Continuous - 24-hr light/0-hr dark.
pH • 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaema*) • 8.1 ± 0.1 (*Skeletonema*) • Measured at beginning and end of test	• pH = 7.7 to 7.8 (0-hr). • pH = 8.1 to 9.1 (96-hr).
Oscillation Rates • 100 cycles/min (*Selenastrum*) • 60 cycles/min (*Skeletonema*)	• Maintained at 100 rpm
Test Containers • 125-500 mL Erlenmeyer flasks • Cleaned/sterilized (solvent and acid) and conditioned • Test solution volume ≤ 50% of flask volume	• 250 mL Erlenmeyer flasks pretreated with Bardac 2280 solution of each respective treatment and plugged with foam stoppers. • 100 mL test solution (<50% of flask volume).

Guideline Criteria	Reported Information
Dilution Water • Sufficient quality (e.g., ASTM Type I) • Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	• River water collected from the Potomac River near White Landing, Maryland. • Primary stock of ^{14}C-DDAC prepared in NANOpure® water.

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test • Water solubility and physical-chemical properties of test chemical determined? • Validated analytical method developed? • Lowest dose at detection limit, upper dose at saturation concentration or 1000 mg/L • If < 50% reduction in growth at highest dose, no definitive test required	• Unknown • Nominal test concentrations were selected in consultation with the Sponsor's Representative and were based upon the results of an exploratory range finding toxicity test. No further details were provided in the Study Report. • A validated analytical method was developed.
Dose Range • 1.5X -2X progression	• 2X progression
Doses • 5 or more concentrations of test substance in a geometric series • > 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	• Six concentrations: Nominal = 13, 25, 50, 100, 200, and 400 µg ai/L. Mean measured (initial) = 10, 20, 39, 82, 174, and 347 µg ai/L. • Percent recovery 78 to 87%. • >90% growth was inhibited (97%).
Controls • Negative and/or solvent each test • Positive - zinc chloride (periodically)	• Negative control • No positive control
Replicates Per Dose • 3 or more (4 or more for *Navicula*)	• 3 replicates per dose and controls.
Duration of Test • 96-hr	• 96-hr.

Guideline Criteria	Reported Information
Growth • Logarithmic growth (controls) by 96-hr or repeat test • 1.5 x 10^6 cells/mL (*Skeletonema*) • 3.5 x 10^6 cells/mL (*Selenastrum*)	• Logarithmic growth in control by 96-hr • Mean of 4.1 x 10^6 cells/mL at 96-hr. in the control. • Increase by factor of 410.
Daily Observations?	• Yes
Method of Observations • Direct - microscopic cell count of at least 400 cells/flask • Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count • Qualitative and descriptive	• Cell counts were performed using an electron particle counter (Coulter Electronics, Inc.). • Cells examined microscopically for atypical morphology. • Growth of cells were assessed for aggregations or flocculation of cells and adherence of cells to the test chamber.
Cell Separation • Syringe ultrasonic bath, or blender; limited sonification (*Anabaena*) • Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	• Rotary shaking.
Algistatic and algicidal effects differentiated?	• The 347 µg ai/L treatment group was maximally inhibited at the end of the 96-hour exposure period. At 96 hours, 0.5 mL aliquots of the test solutions were diluted in 100 mL of river water algal medium and growth was observed. After four days algal growth was sufficient to indicate that the cells had recovered from the effects of the test substance; therefore, effects were found to be algistatic, rather than algicidal.
Maximum Labeled Rate	• Not reported.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes • Original algal cultures obtained from the University of Toronto Culture Collection of Algae and Cyanobacteria and maintained at Wildlife International, Ltd.
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	• Yes
Growth in controls reported?	• Yes
Description of test system and test design included?	• Yes
Initial and final chemical concentrations and pH measured?	• Yes
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	• Yes • Yes
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	• Yes
Raw data included?	• Partial
Methods and data records reported?	• Partial
Statistical Analysis • Mean and standard deviation calculated and plotted? • Goodness-of-fit determined?	 • Only mean calculated and plotted. • Yes

Dose Response

Cell Density (cells/mL)

Mean Measured Concentration at Test Initiation (µg ai/L)	24 -hour		48-hour		72-hour		96-hour	
	Mean Cell Density	Percent Inhibition[a]	Mean Cell Density	Percent Inhibition[a]	Mean Cell Density	Percent Inhibition[a]	Mean Cell Density	Percent Inhibition[a]
Control	34,617	---	168,549	---	844,776	---	4,079,122	--
10	28, 327	18	149,773	11	709,409	16	3,154,346	23
20	32,627	5.7	188,997	-12	981,872	-16	3,686,806	9.6
39	32,842	5.1	180,826	-7	971,170	-15	3,947,144	3.2
82	32,982	4.7	137,486	18	617,271	27	3,115,458	24
174	24,989*	28	74,507*	56	298,437*	65	1,475,998*	64
347	8,917*	74	17,866*	89	32,535*	96	125,211*	97

a) Percent inhibition was calculated relative to the negative control replicates using SAS Version 8.
* Statistically significant difference ($p<0.05$) from the negative control replicates using Dunnett's test.

Mean Area Under the Growth Curve

Mean Measured Concentration at Test Initiation (µg ai/L)	24 -hour		48-hour		72-hour		96-hour	
	Mean Area	Percent Inhibition[a]	Mean Area	Percent Inhibition[a]	Mean Area	Percent Inhibition[a]	Mean Area	Percent Inhibition[a]
Control	295,408	---	2,493,300	--	14,413,300	---	73,260,080	---
10	219,928	26	2,177,132	15	12,187,316	15	58,312,372	20
20	271,528	8.1	2,691,024	-7.9	16,501,452	-14	72,285,580	1.3
39	274,108	7.2	2,598,128	-4.2	16,182,080	-12	74,961,844	-2.3
82	275,784	6.6	2,081,400	17	10,898,484	24	55,451,236	24
174	179,864*	39	1,133,812*	55	5,369,140*	63	26,422,356*	64
347	7,672*	97	89,068*	96	453,876*	97	2,106,820*	97

a) Percent inhibition was calculated relative to the negative control replicates using SAS Version 8.
* Statistically significant difference ($p<0.05$) from the negative control replicates using Dunnett's test.

Statistical Results

Statistical Method: Cell density and area under the growth curve were analyzed statistically by non-linear regression versus concentration to determine EC_{10}, EC_{50}, and EC_{90} values and 95% confidence limits for each 24-hour exposure interval. In cases where EC50 values could not be

determined by non-linear regression, EC values and 95% confidence limits were calculated by linear interpolation versus concentration using TOXSTAT Version 3.5. To determine the NOEC, cell density and the area under the growth curve data were first evaluated for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively, and were compared to the negative control using analysis of variance (ANOVA) and Dunnett's test. **Results are reported based on 0-hour measured concentrations, which are significantly greater than the 96-hour measured concentrations, due to binding of DDAC to the glassware used in the study,**

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Cell Density Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	EC_{90} (95% CI)	NOEC
24-hr	134 (90 - 199)	257 (219 - 300)	>347 (NA)	82
48-hr	67 (44 - 102)	154 (126 - 188)	354 (290 - 433)	82
72-hr	62 (39 - 98)	130 (103 - 165)	274 (223 - 336)	82
96-hr	<10 (NA)	151 (129 - 176)	276 (241 - 316)	82

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Area Under the Growth Curve Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	EC_{90} (95% CI)	NOEC
24-hr	89 (0-166)	257 (203-299)	>347	82
48-hr	93 (68-129)	163 (138-192)	285 (246 - 332)	82
72-hr	69 (45-104)	137 (110-169)	272 (226-327)	82
96-hr	77 (55-107)	145 (122-171)	272 (235-315)	82

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: EC values were calculated by probit linear regression, using the TOXANAL program.

NOEC Determination

The data were first checked for normality using the Chi Square Test, and for homogeneity of variance using the Harley Test or Bartlett's Test. All cell density data were normally distributed and had homogeneous variance. Twenty-four and 96-hour area-under-the-growth-curve data were normally distributed and had homogeneity of variance; 48-hour and 72-hour were normally distributed, but did not have homogeneity of variance, and were square-root transformed in order to meet the requirements for ANOVA. All data were analyzed using ANOVA with Dunnet's test, and William's Test to determine the NOEC. **The 96-hour measured concentrations were used.**

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Cell Density Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	Slope	NOEC
24-hr	15.3 (0 - 95)	204.6 (36 - infinity)	1.13	36.5
48-hr	20.1 (0 - 65)	89.7 (0 - infinity)	1.95	36.5
72-hr	15.1 (0 - 52)	67.9 (0 - infinity)	1.95	27
96-hr	9.64 (0 - 34)	62.2 (0- infinity)	1.57	27

EC_{10}, EC_{50}, and EC_{90} Values (µg a.i./L) for Area Under the Growth Curve Over the 96-hr Period

Time	EC_{10} (95% CI)	EC_{50} (95% CI)	Slope	NOEC
24-hr	10.4 (0 - 68)	97.6 (0 - infinity)	1.31	36.5
48-hr	17.8 (0 - 78)	70.1 (0 - infinity)	1.90	36.5
72-hr	16.2 (0 - 59)	70.1 (0 - infinity)	2.00	27
96-hr	13.6 (70 - 83)	67.5 (0 - infinity)	1.83	27

14. REVIEWER'S COMMENTS:

- At the 96-hour interval, the mean measured concentrations based on liquid scintillation counting (LSC) were 50, 60, 50, 57, 48, and 52 percent of nominal in the 13, 25, 50, 100, 200, and 400 µg/L treatment groups, respectively. Some of the DDAC may have bonded to the glassware. According to the Study Report, DDAC bound to the glass surfaces of the glassware remains biologically available to algae in the test system; however, the Agency will use endpoints calculated on the 96-hour mean measured concentrations.

Kat DDAC sel cap 96h cell dens

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
255	100	97	97	0
101	100	64	64	0
36.5	100	24	24	0
27	100	3	3	0
10.1	100	10	10	0
6.19	100	23	23	0

THE BINOMIAL TEST SHOWS THAT 36.5 AND 101 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 71.3212

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.012751	73.1987	66.53928	80.75105

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.189956	26.47896	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.568319
95 PERCENT CONFIDENCE LIMITS =-.1424836 AND 3.279122

LC50 = 62.23865
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 9.644416
95 PERCENT CONFIDENCE LIMITS = 0 AND 34.21177

TITLE: DDAC sel cap river water 96h cellden
FILE: ddaccdt
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	3686935.0000	3686935.0000
1	control	2	4638900.0000	4638900.0000
1	control	3	3911531.0000	3911531.0000
2	6.19	1	3504285.0000	3504285.0000
2	6.19	2	3141247.0000	3141247.0000
2	6.19	3	2817505.0000	2817505.0000
3	10.1	1	3783043.0000	3783043.0000
3	10.1	2	4478936.0000	4478936.0000
3	10.1	3	2798438.0000	2798438.0000
4	27	1	3224557.0000	3224557.0000
4	27	2	4413270.0000	4413270.0000
4	27	3	4203604.0000	4203604.0000
5	36.5	1	3253189.0000	3253189.0000
5	36.5	2	2768643.0000	2768643.0000
5	36.5	3	3324543.0000	3324543.0000
6	101	1	1589302.0000	1589302.0000
6	101	2	1335014.0000	1335014.0000
6	101	3	1503677.0000	1503677.0000
7	255	1	142876.0000	142876.0000
7	255	2	90743.0000	90743.0000
7	255	3	142013.0000	142013.0000

NOEC - controls - 27
ANOVA + Tukey - 36

DDAC sel cap river water 96h cellden
File: ddaccdt Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	3686935.000	4638900.000	4079122.000
2	6.19	3	2817505.000	3504285.000	3154345.667
3	10.1	3	2798438.000	4478936.000	3686805.667
4	27	3	3224557.000	4413270.000	3947143.667
5	36.5	3	2768643.000	3324543.000	3115458.333
6	101	3	1335014.000	1589302.000	1475997.667
7	255	3	90743.000	142876.000	125210.667

DDAC sel cap river water 96h cellden
File: ddaccdt Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM

1	control	247624397767.000	497618.727	287300.306
2	6.19	118045373401.375	343577.318	198364.457
3	10.1	712964600247.000	844372.311	487498.581
4	27	402588576022.500	634498.681	366327.984
5	36.5	91483504905.375	302462.402	174626.749
6	101	16740205856.328	129383.947	74699.857
7	255	891201226.333	29852.994	17235.634

DDAC sel cap river water 96h cellden
File: ddaccdt Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	38613104253696.000	6435517375616.000	28.326
Within (Error)	14	3180675719424.000	227191122816.000	
Total	20	41793779973120.000		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

DDAC sel cap river water 96h cellden
File: ddaccdt Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	4079122.000	4079122.000		
2	6.19	3154345.667	3154345.667	2.376	
3	10.1	3686805.667	3686805.667	1.008	
4	27	3947143.667	3947143.667	0.339	
5	36.5	3115458.333	3115458.333	2.476	
6	101	1475997.667	1475997.667	6.689	*
7	255	125210.667	125210.667	10.160	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

DDAC sel cap river water 96h cellden
File: ddaccdt Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL

1	control	3	.	.	.
2	6.19	3	984624.347	24.1	924776.333
3	10.1	3	984624.347	24.1	392316.333
4	27	3	984624.347	24.1	131978.333
5	36.5	3	984624.347	24.1	963663.667
6	101	3	984624.347	24.1	2603124.333
7	255	3	984624.347	24.1	3953911.333

DDAC sel cap river water 96h cellden
File: ddaccdt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	4079122.000	4079122.000	4079122.000
2	6.19	3	3154345.667	3154345.667	3596098.333
3	10.1	3	3686805.667	3686805.667	3596098.333
4	27	3	3947143.667	3947143.667	3596098.333
5	36.5	3	3115458.333	3115458.333	3115458.333
6	101	3	1475997.667	1475997.667	1475997.667
7	255	3	125210.667	125210.667	125210.667

DDAC sel cap river water 96h cellden
File: ddaccdt Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	4079122.000				
6.19	3596098.333	1.241		1.76	k= 1, v=14
10.1	3596098.333	1.241		1.85	k= 2, v=14
27	3596098.333	1.241		1.88	k= 3, v=14
36.5	3115458.333	2.476	*	1.89	k= 4, v=14
101	1475997.667	6.689	*	1.90	k= 5, v=14
255	125210.667	10.160	*	1.91	k= 6, v=14

s = 476645.699
Note: df used for table values are approximate when v > 20.

96 h area under the growth curve

Kat DDAC ddac sel cap 96 h area under growth

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
255	100	97	97	0
101	100	64	64	0
36.5	100	24	24	0
27	100	0	0	0
10.1	100	1	1	0
6.19	100	20	20	0

THE BINOMIAL TEST SHOWS THAT 36.5 AND 101 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 71.3212

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.017541E-02	75.75656	69.55574	82.75318

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	1.531962	37.91409	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.822992
95 PERCENT CONFIDENCE LIMITS =-.4333699 AND 4.079353

LC50 = 67.49804
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 13.5717
95 PERCENT CONFIDENCE LIMITS = 0 AND 52.4908

TITLE: ddac sel cap 96h area under gr
FILE: ddacagct
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	68000028.0000	68000028.0000
1	control	2	83861808.0000	83861808.0000
1	control	3	67918404.0000	67918404.0000
2	6.19	1	65523924.0000	65523924.0000
2	6.19	2	57101268.0000	57101268.0000
2	6.19	3	52311924.0000	52311924.0000
3	10.1	1	76195836.0000	76195836.0000
3	10.1	2	87111096.0000	87111096.0000
3	10.1	3	53549808.0000	53549808.0000
4	27	1	59703108.0000	59703108.0000
4	27	2	86143728.0000	86143728.0000
4	27	3	79038696.0000	79038696.0000
5	36.5	1	59123652.0000	59123652.0000
5	36.5	2	48857124.0000	48857124.0000
5	36.5	3	58372932.0000	58372932.0000
6	101	1	28175112.0000	28175112.0000
6	101	2	25335720.0000	25335720.0000
6	101	3	25756236.0000	25756236.0000
7	255	1	2350440.0000	2350440.0000
7	255	2	1582632.0000	1582632.0000
7	255	3	2387388.0000	2387388.0000

NOEC -

ddac sel cap 96h area under gr
File: ddacagct Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	67918404.0	0083861808.0	0073260080.000
2	6.19	3	52311924.0	0065523924.0	0058312372.000
3	10.1	3	53549808.0	0087111096.0	0072285580.000
4	27	3	59703108.0	0086143728.0	0074961844.000
5	36.5	3	48857124.0	0059123652.0	0055451236.000
6	101	3	25335720.0	0028175112.0	0026422356.000
7	255	3	1582632.0	002387388.0	0002106820.000

ddac sel cap 96h area under gr
File: ddacagct Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM

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 1          control84299143058816.0009181456.4785300916.369
 2             6.1944739315674112.0006688745.4483861748.985
 3             10.1293057589543936.00017118924.8959883615.896
 4               27187242138168576.00013683644.9157900256.075
 5             36.532752629931008.0005722991.3453304170.593
 6              1012348323623216.0001532424.100 884745.467
 7              255206421083184.000 454335.870 262310.937
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ddac sel cap 96h area under gr
File: ddacagct Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between		613757516325584896.000229291938759884 8.000		24.898
Within (Error)		141289291122212864.00092092223015168.000		
Total		2015046807447797760.000		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

ddac sel cap 96h area under gr
File: ddacagct Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	73260080.000	73260080.000		
2	6.1	958312372.000	58312372.000	1.908	
3	10.1	72285580.000	72285580.000	0.124	
4		2774961844.000	74961844.000	-0.217	
5	36.5	55451236.000	55451236.000	2.273	
6		10126422356.000	26422356.000	5.978	*
7		255 2106820.000	2106820.000	9.081	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

ddac sel cap 96h area under gr
File: ddacagct Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL

1	control	3			
2	6.19	3	19823775.461	27.1	14947708.000
3	10.1	3	19823775.461	27.1	974500.000
4	27	3	19823775.461	27.1	-1701764.000
5	36.5	3	19823775.461	27.1	17808844.000
6	101	3	19823775.461	27.1	46837724.000
7	255	3	19823775.461	27.1	71153260.000

ddac sel cap 96h area under gr
File: ddacagct Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control		373260080.000	73260080.000	73260080.000
2	6.19		358312372.000	58312372.000	68519932.000
3	10.1		372285580.000	72285580.000	68519932.000
4	27		374961844.000	74961844.000	68519932.000
5	36.5		355451236.000	55451236.000	55451236.000
6	101		326422356.000	26422356.000	26422356.000
7	255		3 2106820.000	2106820.000	2106820.000

ddac sel cap 96h area under gr
File: ddacagct Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	73260080.000				
6.19	68519932.000	0.605		1.76	k= 1, v=14
10.1	68519932.000	0.605		1.85	k= 2, v=14
27	68519932.000	0.605		1.88	k= 3, v=14
36.5	55451236.000	2.273	*	1.89	k= 4, v=14
101	26422356.000	5.978	*	1.90	k= 5, v=14
255	2106820.000	9.081	*	1.91	k= 6, v=14

s = 9596469.299
Note: df used for table values are approximate when v > 20.